# Analysis of Blue Chamomile Essential Oil produced by multi-solvent Solvent Extraction Clevenger Distillation Method

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**ABSTRACT**

Blue colored essential oil samples were obtained from chamomile flowers by a Solvent Extraction Clevenger Distillation (SECD) method and by the standard Clevenger distillation of Chamomile flowers. The solvents used were hexane, acetone, dichloromethane (DCM), ethyl acetate and methanol. The solvents were evaluated in terms of the yield of extract, and the quality of extract (determined by Gas Chromatography-Mass Spectrometry). Of all the SECD extracts, the DCM extract gave the highest yield of the essential oil while the hexane extract gave the lowest yield of the blue essential oil.


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## 1. Introduction

Essential oils are odorous and volatile compounds stored in plants in special brittle secretory structures, such as glands, secretory hairs, secretory ducts, secretory cavities or resin ducts. They are volatile when exposed to heat as compared to other type of oils known as fixed oils. Essential oils mostly exist in plants which have fragrant flowers, leaves, wood, bark, roots, or seeds.

The most common methods of extraction of essential oils use hydrodistillation, Cold Pressing, Solvent Extraction. Newer methods include Turbodistillation, Hydrodiffusion, and Supercritical Carbon Dioxide Extraction. Hydrodistillation can be achieved by one of the two methods:

- Clevenger distillation - the material to be extracted is immersed in water, which is then boiled.
- Steam distillation - steam passes through a bed of the material to be extracted.

Clevenger distillation is used for extraction of essential oils on laboratory scale where plant material can fit into a round-bottomed distillation flask while steam distillation is used on industrial scale and requires special apparatus that can withstand high pressures due to the presence of superheated steam. Also, the movement of steam over the ground flowers in an industrial plant is inefficient hence a huge mass of chamomile flowers has to be utilized to obtain substantial amount of chamomile oil since the yield is often less than approximately 1%.

Mwaniki and Mbugua [1] introduced a method that combined solvent extraction and Clevenger distillation in obtaining an extract similar to the blue oil from steam distillation. Using the solvent acetone to extract the organic components present in the flowers by soaking them for a period of 8 days and concentrating the crude extract which was then transferred to a Clevenger apparatus and steam distillation carried out for 6 hours. The quality of the blue essential oil was comparable (from NMR and GC analysis) with that from direct steam distillation of chamomile flowers.

The SECD method of obtaining chamomile essential oil is a much less expensive method compared to that of direct steam distillation of chamomile flowers because on industrial scale, expensive stainless steel equipment that can withstand superheated steam is used. Additionally, on an industrial scale, chamomile flowers have to be packed on shelves which allow steam to pass through to avoid the flowers compacting like mashed potatoes thereby restricting the movement of steam. All these restrictions are avoided in SECD as steam distillation is carried out on the organic extract using normal glass distillation equipment.

The word chamomile is derived from two Greek words - *chamos* (ground) and *melos* (apple), due to the fact that the plant grows low to the ground, and the fresh flowers have a pleasing apple-scent. Chamomile is a common name for three plants, all being members of the daisy family (Asteraceae compositae); German Chamomile (*Matricaria recutita*, *Chamomilla recutita*, *Matricaria chamomilla*, *Chamomilla recutita* (L.) Rauschert), Roman/English Chamomile (*Chamaemelum nobile*, *Anthemis nobilis*) and Moroccan chamomile (*Ormenis multicaulis, Ormenis mixta* or *Anthemis mixta*). The German Chamomile is considered the most potent of the three and has received more scientific evaluation [2-28].

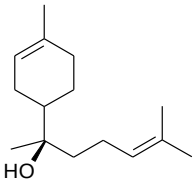
Fig 1a

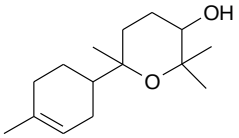
Fig 1b

**Fig 1: Chamomile flowers from University of Nairobi farm at Kibwezi**

The chamomile plant contains a number of components which include *alpha*-bisabolol **1**, bisabolol oxides A & B (**2-3**), Farnesene **4**, Matricine **5** (a sesquiterpene lactone), Coumarins (herniarin **6** and umbelliferone **7**), Flavonoids (apigenin **8**, quercetin **9**, patulein **10**, luteolin **11**), cis and trans-en-yn-dicycloethers **12-13,** Spathulenol **15** and *t*-Cardinol **16**. During the distillation of the flowers, matricine **5** present in the flowers is converted to the hydrocarbon chamazulene **14** (containing five conjugated double bonds) as shown in Figure **1**. It is chamazulene that is responsible for the inky blue colour of the essential oil.

Over 120 constituents have been identified in chamomile including 28 terpenoids, 36 flavonoids, and 52 additional compounds with potential pharmacological activity [29].


(-)-α-Bisabolol
**1**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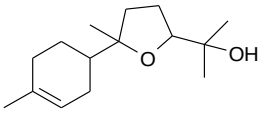
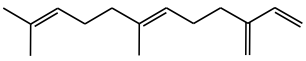
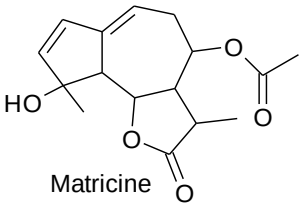
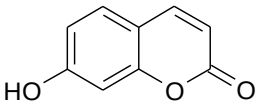
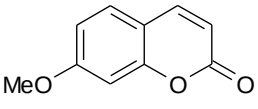
**2**

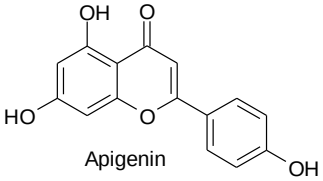

(-)-α-Bisaboloxide B
**3**

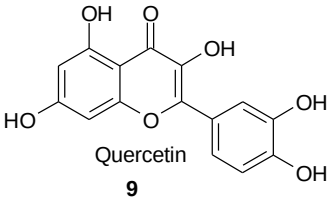

β-Farnesene
**4**

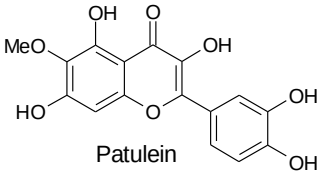

Matricine
**5**

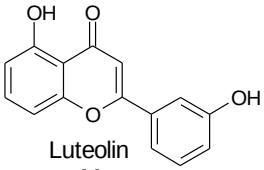

Umbelliferone
**6**

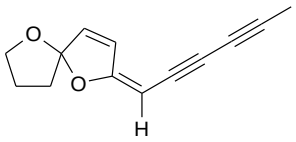

Herniarine
**7**

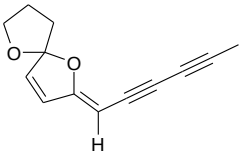

Apigenin
**8**

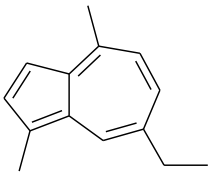

Quercetin
**9**

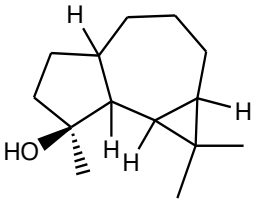

Patulein
**10**

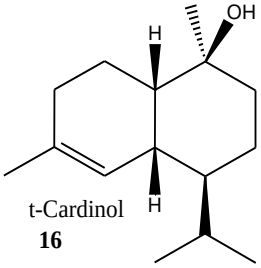

Luteolin
**11**

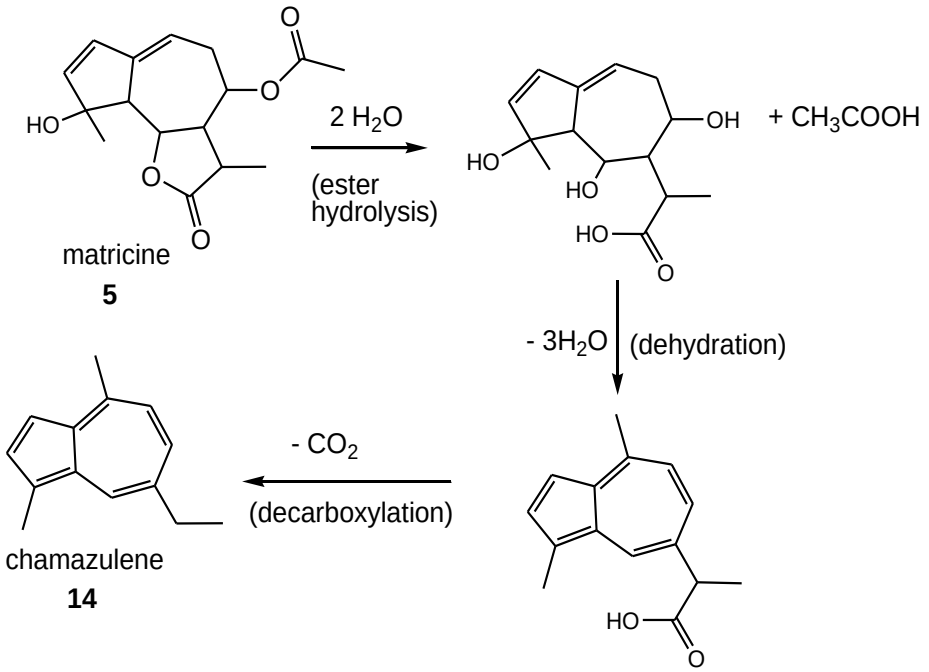

cis Enyne dicyclo ether
**12**


trans Enyne dicyclo ether
**13**


chamazulene
**14**


Spathulenol
**15**


t-Cardinol
**16**




**Scheme 1: Conversion of matricine (present in flowers) to the highly conjugated blue coloured chamazule**

**Figure 2: Blue Chamomile essential oil**

Chamomile herb is unique due to the medicinal value exhibited by its components. The flavonoid Apigenin **8** exhibits a wide range of factors: has anti-cancer activity [30-35], antiviral activity [36-37], antispasmodic activity [38] and sedative activity [39]. In general, the flavonoids present in the chamomile extract have been shown to have anti-inflammatory activity [40]. The optically active alcohol *alpha*-bisabolol **1** is antiphlogistic [41], antiulcerogenic [42] and promotes granulation and tissue regeneration and thereby shortens the healing time for burns [43]. Matricine **5** has anti-inflammatory activity [44] and its steam decomposition product, chamazulene **14**, has been shown to have the following medicinal actions: anti-inflammatory and [45] anti-oxidant activity [46-48]. The cyclic spiro-ethers (**12-13**) on the other hand have antimicrobial, anti-inflammatory, anti-anaphylactic and spasmolytic properties [49-50].

Chamomile tea (made from chamomile flowers) has anti-oxidant activity, [51] Gastrointestinal effects [52], in addition to the sedative effect [53]. The most enhanced medicinal value is obtained when all the components are present in the extract [54-64]. As a result of these activities, the chamomile extract has found applications in treating various ailments. A number of products where Chamomile has been incorporated are available in the market. These include: Cosmetic cream (protection and nurturing the skin-sensitive and dry, irritating skin, improves blood circulation and skin regeneration), Herbal balm (treatment of skin wounds) Gel (astringent, reduces pain and irritation), Lipstick (For irritated lip skin), Hand Cream (Protects hands and nails), Emollient foot cream (For rough dry skin) Shampoo (For thin and sensitive hair and for sensitive skin), Cosmetic soap, Wet towels (Cleans and protects baby skin), Toothpaste (soothes inflammation, strengthens the gums and protects the teeth), Mouth wash (used against bleeding gums and mouth infections), Cosmetic tonic (Bactericidal, against acne and for problematic young skin), Cosmetic facial cream (against wrinkles, balances skin moisture, nurtures), Bath salt (For sensitive, tired and irritated skin), Bath gel (for sensitive skin), Herbal alcohol solution (Against stomach problems, gum problems and diarrhea), Chamomile Instant tea (for babies from 1$^{st}$ week) and Herbal tea (tranquilizing herbal mix, herbal mix for patients with gall stones, against cellulite, for slimming, against insomnia, for kidney and gall-bladder, against menstrual problems, for purification, for prostate gland and for breast-feeding mothers).

## 2. Methodology

### a) Materials

The solvents used were of general purpose grade but distilled before use, in order to remove any impurities. HPLC grade *n*-hexane was used for GC-MS analysis. Also, the drying agent (Anhydrous sodium sulphate crystals) used was general purpose grade.

Chamomile flowers obtained from Kangari, Murang'a County, in central Kenya, were used in this research. The flowers were crushed and sieved thoroughly to obtain the finest material.

### b) Solvent extraction-Clevenger distillation (SECD) using different solvents

20g of ground chamomile flowers were placed in a conical flask and 200ml of distilled acetone added. The flask was sealed with an Aluminum foil and kept un-agitated for 8days. The procedure was now repeated using the same mass of flowers but using different solvents i.e., DCM, Ethyl-acetate, hexane, hexane: acetone (1:1) and methanol. After the duration, the mixtures were filtered and the filtrate concentrated using rotary evaporator under reduced pressure. Boiling chips were also placed into the 500 ml round bottom flask and a Clevenger apparatus together with a condenser were attached and distillation carried out for 6 hours. The final distillate was dried using anhydrous sodium sulphate and its weight determined after which it was stored in a refrigerator.

### c) Standard Clevenger distillation

20g sample of ground chamomile flowers was transferred into a distillation flask and 250ml of distilled water added. The extraction process was carried out for six hours after which the essential oil was that collected on the graduated column of the Clevenger apparatus was diluted with 20ml hexane and the resulting blue organic extract drained into a 50ml conical flask. Anhydrous magnesium sulphate was added to remove traces of water in the extract, and the hexane solvent removed under reduced pressure on a rotary evaporator, but without heating the water bath to minimize the loss of volatile components of the chamomile essential oil.. The mass of the blue oil was recorded.

### (d) GC-MS Analysis

All the SECD extracts were analysed by GC-MS. About 5 mg of each sample was dissolved in 1 ml HPLC grade *n*-hexane. At the GC-MS 1 µL of the sample solution was injected. Analysis was done using an Agilent Technologies 6890N Network GC system with a 5975 Inert XL Mass Selective Detector and a 7683B Series Injector. The column used was a ZB-5MSi which was 30 m long, had 0.25 mm internal diameter and 0.25 µm film thickness. The carrier gas was Helium with the split ratio 1:8 and flow rate 1ml/min (37cm/s). The injector temperature was $280^0$C and the MS source temperature was $200^0$C. The MS detector was operated in the Electron Impact mode 70 eV at a scan rate of 2 scans/sec with an acquisition mass range of 40-500 amu. The temperature programming was $60^0$C for 1 minute then up to $110^0$C at $10^0$C/min. The temperature was then held for 2 min at $110^0$C, then up to $250^0$C at $10^0$C/min and then held at $250^0$C for 13 minutes.

## 3. Results and discussion

### (a) Yield of Chamomile Oil

The yields for both the SE-CD and Standard Clevenger methods are shown in Table 1 below.

**Table 1: Yield of chamomile essential oil using SECD**

| Name of Solvent used in SECD | Volume of solvent(ml) | Mass of Chamomile flowers used (g) | Percentage yield of chamomile essential Oil (%) |
|---|---|---|---|
| Hexane | 200 | 20 | 0.69 |
| Acetone | 200 | 20 | 0.90 |
| Ethyl acetate | 200 | 20 | 0.72 |
| Hexane/Acetone | 200 | 20 | 0.92 |
| Methanol | 200 | 20 | 0.87 |
| DCM | 200 | 20 | 0.93 |
| Standard Clevenger method | 200 | 20 | 0.97 |

The DCM extract gave the highest yield of chamomile oil compared to other SECD extracts while the Hexane extract gave the lowest yield of the chamomile essential oil.

Standard Clevenger distillation of Chamomile flowers utilized 20 g of chamomile flowers and gave a yield of 0.97% (approx. 1%) of the essential oil. This percentage is higher than the highest produced by any of the SECD method. It appears the solvent extraction aspect of the SECD method leads to a moderate loss in yield. However, as mentioned in the literature, on industrial scale, the economic benefits of SECD by far outweigh those of the steam distillation method. It was pointed out in an earlier publication [1] that the gas chromatograph of the SECD method using acetone as the solvent produced similar components to standard Clevenger method but with varying concentrations.

**Table 2: Percentages of the main components in G.C of chamomile SECD extracts.**

| **Component** | **Retention Time (min)** | **Percentage by Peak Area** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **Hex** | **H/Ace** | **Ace** | **DCM** | **EA** | **E/D** | **Meth** |
| β-farnesene | 13.988 | 3.602 | 3.462 | 1.323 | 6.326 | 4.123 | 1.930 | 3.763 |
| Spathulenol | 15.774 | 0.555 | 0.570 | 0.607 | 0.977 | 0.934 | 0.816 | 0.725 |
| T-cadinol | 16.533 | 0.789 | 0.941 | 1.784 | 1.561 | 2.293 | 1.893 | 0.890 |
| α-Bisabolol oxide B | 16.697 | 3.063 | 5.904 | 4.471 | 4.860 | 5.894 | 5.302 | 5.713 |
| α-Bisabolol | 16.994 | - | 1.773 | 2.589 | - | - | - | 2.198 |
| Chamazulene | 17.641 | 0.859 | 2.390 | 1.758 | 1.328 | 1.744 | 1.654 | 1.680 |
| Bisabolol oxide A | 17.814 | 38.548 | 68.474 | 67.787 | 62.431 | 55.974 | 64.197 | 65.831 |
| Cis-enyne | 19.189 | 0.681 | 0.641 | - | 0.685 | - | - | 1.788 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| dicycloether | | | | | | | | |
| Trans-enyne dicycloether | 19.285 | 1.021 | 0.684 | - | 0.704 | - | - | 0.916 |

**Hex**-hexane, **H/Ace**-hexane/acetone (1:1), **Ace**-acetone, **EA**-ethyl acetate, **E/D**-ethyl acetate/DCM (1:1), **Meth**-methanol, - Not detected.

Only in 3 of the 7 SECD extracts was α-bisabolol detected. These were the hexane/acetone, acetone and methanol extracts. The acetone extract has the highest percentage of α-bisabolol at 2.59% while the lowest was in the hexane/acetone extract at 1.77%. The hexane/acetone SECD extract has the highest percentage of chamazulene at 2.39% while the hexane extract has the lowest at 0.86%. The percentage of bisabolol oxide A was highest in the hexane/acetone SECD extract at 68.5%, followed by the acetone extract at 67.8%. The hexane extract again had the lowest percentage of bisabolol oxide A at 38.6%. Overall, the polar solvents performed better than the non-polar solvents in extracting the main components of chamomile. The percentages of the main components in the standard Clevenger method are similar to that of the acetone SECD method. This similarity was pointed out in the first report on the SECD method [1].

The yield of the extract was comparable to that of Clevenger distillation and GC-MS analysis of the extract revealed that the quantity of chamazulene was 4% higher than that from Clevenger distillation method. This was probably so because the use of the relatively non-toxic moderately polar acetone solvent, rather than water, extracts more matricin from chamomile flowers which is subsequently transformed to chamazulene in the boiling water temperature of Clevenger distillation. The GC chromatograms also showed that the two extracts had varying amounts of components from their varying peak heights.

GC-MS analysis of the SECD extracts of different solvents revealed that they have varying amounts of the main components (Table 2). The DCM SECD extract has the highest percentage of β-farnesene at 6.33% and the acetone extract the lowest at 1.32%. Spathulenol on the other hand was highest in the DCM extract at 0.98% and lowest in the hexane extract at 0.56%. α-bisabolol oxide B was highest in the hexane/acetone and ethyl acetate SECD extracts at 5.9% and 5.9% respectively. The hexane extract had the least percentage at 3.06%.

## 4. Conclusion

This work extended the previous work on SECD [1] that focused only on acetone as solvent and has made a comparison of a number of solvents available in the laboratory. Given the fact that large quantities of flowers can be placed in a drum for solvent extraction after 8 days and thereafter Clevenger distillation after concentrating the crude, this approach using different solvents opens a door for a number of constituents of the chamomile essential oil which could not be available cheaply on large scale due to the cost of steam equipment to be used in a number of products other than in aromatherapy. For all practical purposes, the yield of essential oil is approximately 1% which is the maximum possible even using the laboratory scale steam distillation of chamomile flowers.

The use of solvents such as dichloromethane implies that the essential oil produced by this method may not be applied in aromatherapy to avoid any traces of solvent being inhaled. This SECD method can be used in a number of products such as chamomile soap, where solvents such

as methanol etc. are actually used in the production of the finished product. Other applications include the making available cheaply various components of the chamomile essential oil (**1-16)** like Chamazulene (**14**) could only be found in small quantities currently for research as G.C. standards, the method described in this work makes them available commercially in good quantities.

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